Potential role of the formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is a progressive, neurodegenerative disease characterized by the presence of multiple senile plaques in the brain tissue, which are also associated with considerable inflammatory infiltrates. Although the precise mechanisms of the pathogenesis of AD remain to be determined, the overproduction and precipitation of a 42 amino acid form of β amyloid ($A\beta_{42}$) in plaques have implicated Aβ in neurodegeneration and proinflammatory responses seen in the AD brain. Our recent studies revealed that the activation of formyl peptide receptor-like 1 (FPRL1), a seven-transmembrane, G-protein-coupled receptor, by $A\beta_{42}$ may be responsible for accumulation and activation of mononuclear phagocytes (monocytes and microglia). We further found that upon binding FPRL1, $A\beta_{42}$ was rapidly internalized into the cytoplasmic compartment in the form of $A\beta_{42}$/FPRL1 complexes. Persistent exposure of FPRL1-expressing cells to $A\beta_{42}$ resulted in intracellular retention of $A\beta_{42}$/FPRL1 complexes and the formation of Congo-red-positive fibrils in mononuclear phagocytes. Our observations suggest that FPRL1 may not only mediate the proinflammatory activity of $A\beta_{42}$ but also actively participate in $A\beta_{42}$ uptake and the resultant fibrillar formation. Therefore, FPRL1 may constitute an additional molecular target for the development of therapeutic agents for AD. *J. Leukoc. Biol.* 72: 628–635; 2002.

Key Words: *NSAID · central nervous system · Aβ · macrophage · microglia*

INTRODUCTION

Leukocytes accumulate at sites of inflammation and microbial infection in response to bacterial and host tissue-derived chemoattractants, which activate cellular receptors with seven-transmembrane (STM) structure and G-protein-coupling characteristics [1–4]. Over the past few years, substantial interest has been generated by the intriguing pathophysiological significance of two STM receptors, originally identified as receptors for the bacterial and synthetic chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLF) [5–7]. In human, the prototype receptor formyl peptide receptor (FPR) is activated by low concentrations (in the picomolar to low nanomolar range) of fMLF and is considered a high affinity fMLF receptor. An FPR variant, FPR-like 1 (FPRL1), interacts with high concentrations (in the micromolar range) of fMLF and is defined as a low affinity fMLF receptor [5–7]. FPR1 and FPR2, the mouse counterparts of human FPR and FPRL1, respectively, have been shown to interact with fMLF with similar pattern as human receptors [8, 9]. FPR and FPRL1 and their murine analogues FPR1 and FPR2 are highly expressed by peripheral blood phagocytic leukocytes. Recent studies have shown that human FPR and FPRL1 could be detected in a variety of cells of the nonhematopoietic origin [7]. Activation of FPR (FPR1) or FPRL1 (FPR2) on the cells by agonists results in a series of signaling events that lead to cell adhesion, chemotaxis, phagocytosis, release of reactive oxygen intermediates, and production of proinflammatory cytokines [5–7]. Despite the fact that the FPRs are among the earliest chemotactic receptors identified and molecularly cloned, the in vivo significance of these receptors remains to be determined. Mice depleted of FPR1 did not show any spontaneous phenotypic defects yet were more susceptible to *Listeria monocytogene* infection [10]. Such mice exhibited impaired neutrophil chemotaxis in response to bacterial fMLF, indicating that this receptor is an active participant in innate host defense against microbial infection. On the other hand, due to the lack of mouse models with disrupted gene coding for FPR2, the counterpart of human FPRL1, it is more difficult to evaluate the in vivo pathophysiological role of this receptor. However, numerous recent studies have identified a great variety of exogenous and host-derived chemotactic agonists for FPRL1. At least three of the FPRL1-specific chemotactic agonists—the serum amyloid A (SAA), the 42 amino acid form of amyloid β ($A\beta_{42}$), and a peptide fragment of the human prion protein (PrP106—126)—are host-derived polypeptides associated with amyloidogenic diseases [11–13]. Thus, FPRL1 may play a significant role in proinflammatory responses seen in systemic amyloidosis, Alzheimer's disease (AD), and prion diseases, in which overproduction of these polypeptides with infiltration of activated mononuclear phagocytes into the sites of lesions is a characteristic feature. These amyloidogenic diseases are associated with a considerable inflammatory involvement at their lesions. In this review, we will discuss the possible contribu-

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tion of FPRL1 to the proinflammatiory aspects of AD and its relevance to $A\beta_{42}$ uptake and fibrillar formation.

Identification of FPRL1 as a functional receptor for $A\beta_{42}$

The first host-derived chemotactic peptide agonist identified for FPRL1 is the acute phase protein SAA [11]. SAA is normally present in serum at 0.1 μM levels, but its concentration is markedly elevated by up to 1000-fold during acute phase responses. The precise pathophysiological role of SAA is not clear. Under normal conditions, SAA is bound to high density lipoprotein and is therefore thought to be a participant in lipid transportation and metabolism. In chronic or recurrent inflammatory conditions, elevated SAA can develop into reactive amyloidosis characterized by deposition of Congo-red positive, birefringent, nonbranching fibrils in peripheral tissues, which may lead to progressive destruction of organ function. In this process, SAA is enzymatically cleaved into fragments that form the basis for amorphous amyloid fibril deposits [14, 15]. As monocytes/macrophages are the source of enzymes that cleave SAA, and such cells accumulate at the sites of amyloid deposits, FPRL1 may serve as a "sensor" for cells to recognize elevated SAA and promote recruitment of inflammatory cells.

The identification of FPRL1 as a functional receptor for SAA prompted us to consider whether FPRL1 might also recognize other host-derived peptides that possess amyloidogenic and proinflammatory activities similar to SAA, despite the divergence in the primary sequences among these molecules. One such candidate is $A\beta_{42}$, which is a key component of the neurodegenerative process of AD. $A\beta_{42}$ is one of the enzymatic cleavage fragments of the amyloid precursor protein (APP), which is a normal constituent of neuronal cells and is thought to be important for neuronal development and function. Mutations in genes encoding APP and the putative APP cleavage enzyme presenilin are associated with increased production of Aβ peptides, including $A\beta_{42}$ and $A\beta_{40}$, by neuronal cells and are associated with familial forms of AD, which are characterized by the early onset of dementia (**Fig. 1**) [16]. In the sporadic form of AD, the precise cause of increased Aβ production in the brain is not clear and may be related to a variety of pathological insults such as atherosclerosis, injury, and infection. As reported, normal aging is also associated with increased production of Aβ peptides in the central nervous system (CNS; **Fig. 1**). The characteristic features of AD are the appearance of multiple senile plaques in brain tissues and a progressive cognitive impairment as a consequence of extensive neuronal loss [16]. A senile plaque is a lesion composed of deposits of $A\beta_{42}$-based amyloid, surrounded and infiltrated by activated microglia [17, 18], which are believed to represent cells of the mononuclear phagocyte lineage in the CNS. In vitro, $A\beta_{42}$ or shorter peptide fragments such as $A\beta_{1-40}$ and $A\beta_{25-35}$ have been reported to activate microglia and blood-derived monocytes, as indicated by increased cell adhesion, chemotaxis, phagocytosis, and production of neurotoxic and proinflammatory mediators [19–22]. In AD patients, chronic inflammatory cellular infiltrates are associated with Aβ deposits in the brain tissues [17, 18]. Some retrospective, epidemiological studies [23] have revealed that for patients treated with nonsteroidal anti-inflammatory drugs (NSAIDs) for diseases

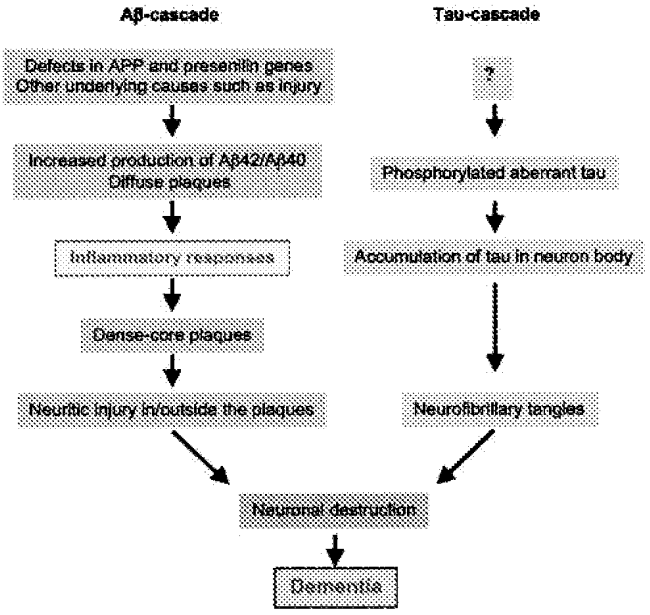

Fig. 1. Schematic representation of the pathogenic process of AD. In familial and sporadic forms of AD, a key feature of the disease is an increased production and accumulation of Aβ peptides, $A\beta_{42}$ in particular, which initially form diffuse plaques in brain tissue. An ensuring, proinflammatory response, triggered by $A\beta_{42}$ and possibly $A\beta_{40}$ as well, may favor the formation of dense-core plaques. Eventual loss of neurons is presumably caused by "neurotoxins" released by microglia and astrocytes, as well as by direct toxicity of deposited $A\beta_{42}$. The aberrant tau is highly phosphorylated and aggregates in the neuronal cell body to promote the formation of neurofibrillary tangles in AD, which also cause neuronal disfunction. The cause for increased tau in AD remains to be defined.

unrelated to AD, such as rheumatoid arthritis, the risk of developing AD was significantly reduced. The effectiveness of NSAID treatment in reducing the risk of AD was also supported by prospective, longitudinal studies [24–26]. In some smaller scale studies, NSAID was found to improve the cognitive abilities, to retard disease progression, and to significantly reduce the number of plaque-associated, reactive microglia in brain tissues of AD patients [27]. In vitro, NSAIDs have been shown to inhibit Aβ-induced mononuclear phagocyte activation and the release of neurotoxins [28]. In a mouse model of human AD-like syndrome, an extended period of oral administration of an anti-inflammatory drug, ibuprofen, reduced AD-like pathology in the brain, including Aβ deposition, cerebral plaque load, plaque-associated microglial activation, and overproduction of the proinflammatory cytokine interleukin (IL)-1 [29]. Therefore, both laboratory and clinical studies support the critical role of inflammation in the progression of AD and the beneficial effect of NSAIDs. Another important pathological feature in AD is the accumulation of a highly phosphorylated and aggregated microtubule binding protein, tau, in the neuronal cell body, which results in neurofibrillary tangles and contributes to the loss of neurons [30] (**Fig. 1**). However, the interrelationship between Aβ cascade and tau-related tangle cascade in the disease process of AD remains unclear, and information is scarce concerning the role of aberrant tau in AD-associated inflammation.

Experimental evidence suggests that $A\beta_{42}$ excerts its proinflammatory and neurotoxic activities through interaction with

specific cell receptor(s). The search for receptor(s) used by $A\beta_{42}$ has yielded several candidate molecules such as the scavenger receptor (SR) [31] and the receptor for advanced glycation end products (RAGE) [32], both of which have been reported to bind $A\beta_{42}$. SR and RAGE are promiscuous cell surface receptors that recognize a diverse array of molecules. Although SR may mediate Aβ-stimulated cell adhesion and phagocytosis of Aβ by mononuclear phagocytes, RAGE was reported to be involved in Aβ-induced microglial chemotaxis and neuronal release of macrophage-colony stimulating factor, which is a proliferative signal for mononuclear phagocytes. However, some studies yielded contrary evidence and suggested the existence of additional cell surface receptors for $A\beta_{42}$. Based on the properties of signal transduction pathways elicited by $A\beta_{42}$ in mononuclear phagocytes, such as induction of calcium mobilization and activation of G-proteins, protein kinase C, as well as tyrosine kinases, the use of STM receptor(s) by $A\beta_{42}$ was postulated [19, 22, 33]. In this regard, the bacterial chemotactic peptide fMLF was shown to attenuate the production of proinflammatory cytokines induced by $A\beta_{42}$ in endotoxin-stimulated rat microglia and a human myeloid cell line THP-1 [34]. Consistent with these observations, increasing concentrations of fMLF progressively desensitized monocyte response to $A\beta_{42}$ in calcium mobilization assays [12]. These results suggested that $A\beta_{42}$ might share a receptor with fMLF, and as high concentrations (in high micromolar range) of fMLF were required to completely abolish the subsequent monocyte response to $A\beta_{42}$, we hypothesized that $A\beta_{42}$ might use a low affinity fMLF receptor. Indeed, in cell lines transfected with the high affinity fMLF receptor FPR, $A\beta_{42}$ only induced a weak calcium flux, but not chemotaxis. In contrast, in HEK293 cells overexpressing the low affinity fMLF receptor FPRL1, $A\beta_{42}$ elicited robust responses in calcium mobilization and cell migration [12]. As directional cell migration in vitro is correlated with chemoattractant-induced cell recruitment in vivo to sites of inflammation and tissue injury, FPRL1 appears to be a pathophysiologically relevant receptor in $A\beta_{42}$-mediated proinflammatory responses of AD. This hypothesis is further supported by our detection of high levels of FPRL1 gene expression by CD11b+ mononuclear phagocytes surrounding and infiltrating the Congo-red positive plaques in brain tissues of AD patients [12]. Human $A\beta_{42}$ has also been identified as a specific chemotactic agonist for FPR2, a murine homologue of human FPRL1, as demonstrated by Tiffany et al. [35]. Considering the difficulties in conducting extensive research in humans, the identification of a murine receptor for $A\beta_{42}$ will facilitate further in vivo studies of the role of FPRL1 with mouse models of AD. Another Aβ peptide, $A\beta_{40}$, has also been implicated in the AD pathogenesis and is an activator of mononuclear phagocytes [21]. Our preliminary study revealed that compared with $A\beta_{42}$, $A\beta_{40}$ was a weaker chemotactic agent and promoted Ca^{2+} flux in monocytic cells and FPRL1-transfected HEK293 cells at high concentrations (greater than 50 μM; Y. Le and W. Gong, unpublished observation). Such results nevertheless suggest that $A\beta_{42}$ and $A\beta_{40}$ may share FPRL1 for their monocytic cell-activating effects.

As prion diseases share some similarities with AD in pathological characteristics, we also investigated the involvement of formyl peptide receptors in the progression of this type of neurodegenerative diseases. Prion diseases affect many mammalian species including human (Creutzfeldt-Jakob disease), sheep (scrapie), and cattle (spongiform encephalopathy or "mad cow disease") [36]. It has been recognized that the etiological agent in these diseases is an aberrant isoform of the cell surface glycoprotein, the prion protein (PrPc) [36]. The pathological isoform of PrPc forms deposits in the extracellular spaces of diseased CNS at sites infiltrated by activated microglia and possibly blood-borne monocytes [37, 38]. Multiple neuritic plaques similar to those seen in AD are present in brains affected by prion diseases, and it is proposed that activation of mononuclear phagocytes is required for the neurotoxicity of prion isoform or its peptide derivatives such as PrP106–126 [38]. PrP106–126 is a 21 amino acid fragment of the human prion protein and has been shown to form fibrils in vitro and to elicit a diverse array of biological responses in mononuclear phagocytes, i.e., monocytes and microglia, including calcium mobilization, protein tyrosine phosphorylation, and production of proinflammatory cytokines [39–42]. Interestingly, recent studies suggest the possible coexistence of prion disease pathology in AD, as brain lesions of some familial AD patients were positively stained by an anti-PrP106–126 antibody, which recognizes the pathologic isoform of prion protein [43]. Our studies have revealed that PrP106–126 also uses FPRL1 as a functional receptor to induce chemotaxis and activation of human mononuclear phagocytes [13]. In addition, PrP106–126 significantly increases the production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and IL-1β by human monocytes [13]. Thus, FPRL1 may also play a role in the proinflammatory aspects of prion diseases.

THE EXPRESSION AND FUNCTION OF FPRL1 IN MICROGLIAL CELLS

Microglial cells are essential components in the development, inflammation, and immunological responses in the CNS. In fact, it has been proposed that there is no pathology in the CNS without active participation of microglia [44, 45]. Microglial cells are considered to be of the mononuclear phagocyte lineage and reside in various areas of CNS during fetal development [44, 45]. Compared with peripheral blood monocytes, microglial cells under normal conditions are at a more quiescent state and do not express high levels of activation markers and lack phagocytic capacity. However, these cells are capable of rapidly reacting to even minor pathological insults in the CNS and become key phagocytic cells engaged in the defense of neuronal parenchyma against infection, inflammation, trauma, ischemia, and tumors [44, 45].

Similar to monocytes and macrophages, microglial cells express a variety of STM chemoattractant receptors that may account for the ability of these cells to migrate and accumulate at sites of inflammation and infection in the CNS. For instance, unstimulated human or rodent microglial cells express the receptors for C5a and a number of chemokines, including CXCR4, and migrate in response to the ligands specific for these receptors in vitro [46–49]. However, the expression and function of the receptors for the chemotactic peptide fMLF are less clear in microglial cells. A limited number of studies

detected the expression of the gene for the high affinity fMLF receptor, FPR, in normal adult human microglia [50, 51], yet the level of receptor protein was reportedly low, and no functional activities were described [50, 51]. It has also been reported that rodent microglia lack the capacity to migrate in response to fMLF [49], suggesting that fMLF receptors in these cells are not expressed or are expressed at a low level. We investigated the expression and function of formyl peptide receptors in a well-established mouse microglial cell line, N9. A low level expression of the genes encoding FPR1 and FPR2, the high and low affinity fMLF receptors, respectively, was detected in N9 cells, but these cells did not respond to chemotactic agonists known for fMLF receptors. Only after incubation with bacterial lipopolysaccharide (LPS) did N9 cells increase the expression of genes for FPR1 and FPR2 and develop a species of specific, low affinity binding sites for radioisotope-labeled fMLF. The LPS-stimulated N9 cells exhibited marked calcium mobilization and chemotaxis in responses to fMLF in a concentration range that typically activates the low affinity receptor FPR2. These cells additionally were chemoattracted by FPR2-specific agonists including a peptide derived from HIV-1 envelope protein and the AD-associated $A\beta_{42}$ [35, 52]. Primary murine-microglial cells isolated from newborn mouse brains also expressed low levels of FPR1 and FPR2 genes under resting conditions and similar to N9 cell line, responded to FPR2-specific peptide agonists only after LPS treatment [52]. The lack of an apparent FPR1-mediated response of microglial cells to low concentrations of fMLF is intriguing. However, this deficiency was similarly observed by an earlier study of rat primary microglial cells, which could be stained positively with an antibody against the human high affinity fMLF receptor FPR but did not respond to fMLF by release of the proinflammatory cytokine IL-1 [34]. On the other hand, only LPS-treated rat microglial cells released IL-1 upon stimulation with $A\beta_{42}$ [34], a specific agonist for human FPRL1 and murine FPR2 [12, 35, 52]. These results suggest that LPS selectively up-regulates the function of the low affinity fMLF receptor in rodent microglial cells. Whether this conclusion is also applicable to human microglial cells remains to be determined.

LPS is a major component of the outer membrane of Gram-negative bacteria and an inducer of host innate response to infection [53]. It is well established that LPS activates phagocytic leukocytes, including microglia, to release proinflammatory mediators. Furthermore, although LPS rapidly increases gene transcription and protein production of a number of cytokines and chemokines, it down-regulates the expression and function of a number of chemokine receptors, including CCR1, CCR2, and CCR5 [54–57] in monocytes or CXCR1 and CXCR2 in neutrophils [58–61]. Such reciprocal up-regulation of the expression of ligands and down-regulation of receptors by LPS have been proposed as a protective host reaction aimed at limiting excessive inflammatory responses. Studies of the mechanisms of down-regulation of chemokine receptors by LPS have shown that LPS reduces chemokine receptor gene transcription or mRNA stability [54, 60, 62]. LPS is also capable of rapidly inducing internalization of the chemokine receptors, presumably by activating protein tyrosine kinases and metalloproteinases [59, 61] without affecting receptor gene expression [55]. This is similar to our observations with murine microglial cells in which LPS treatment markedly reduced surface expression of the binding sites for the chemokine stromal cell-derived factor-1 (SDF-1)α and abolished cell migration to SDF-1α without a substantial effect on the expression of mRNA for the receptor CXCR4 [52].

The effect of LPS on the expression and function of fMLF receptors is rather complicated and may be cell-type-dependent. For instance, in neutrophils, LPS primes the cell response to fMLF, possibly by increasing the surface expression of the intracellularly stored receptor pool [63–66], whereas in monocytes, LPS decreases the cell response to fMLF, presumably by down-regulation of the receptor gene [54, 67, 68]. In murine-microglial cells, LPS clearly increased the expression of the FPR2 gene, and this effect of LPS was not diminished by addition of neutralizing antibodies against TNF-α and IL-1, suggesting that stimulation of the cells by LPS is independent of the production of proinflammatory cytokines [52]. However, we have found that TNF-α by itself is also able to up-regulate the expression and function of FPR2 with concomitant down-regulation of CXCR4 in murine-microglial cells [69]. These results suggest that FPR2 in murine microglial cells can be selectively up-regulated by bacteria and host-derived proinflammatory signals, which may have considerable biological significance in disease states in the CNS. The low responsiveness of unstimulated microglial cells to FPR2 agonists may be important for the homeostasis of the CNS, which under normal conditions, is protected by the blood-brain-barrier (BBB) and is not readily exposed to pathogens. However, in experimental endotoxemia, LPS was reported to enter the brain parenchyma by diffusion through specific regions in the brain, where unique structures of microvessels form incomplete BBB [70]. This leakiness in BBB enables systemically circulating LPS to stimulate brain cells including microglia. Conversely, TNF-α is elevated in a variety of CNS diseases associated with inflammation, including AD. Therefore, microglial cells, by responding to the bacterial signal LPS or endogenous TNF-α, may become activated to assume the full characteristics resembling tissue macrophages, including the enhancement of the FPR2 function. Such a "gain of function" by microglial cells may facilitate their accumulation at sites of aberrant increases in the production of host-derived and bacterial chemotactic agonists. In this context, the concomitant down-regulation by proinflammatory signals LPS and TNF-α of microglial cell responses to SDF-1α—a chemokine mainly implicated in hematopoiesis and development [3, 4] in favor of mobilization of the cells toward proinflammatory chemoattractants such as ligands for FPR2/FPRL1—results in amplifying their response to agonists associated with neurodegenerative diseases.

THE ROLE OF FPRL1 IN $A\beta_{42}$ UPTAKE AND FIBRILLAR FORMATION

In amyloid precursor protein transgenic mice, microglial cells accumulate in greater numbers around amyloid-containing neuritic plaques than diffuse plaques [71]. Several studies on the association of monocytic phagocytes with various stages of

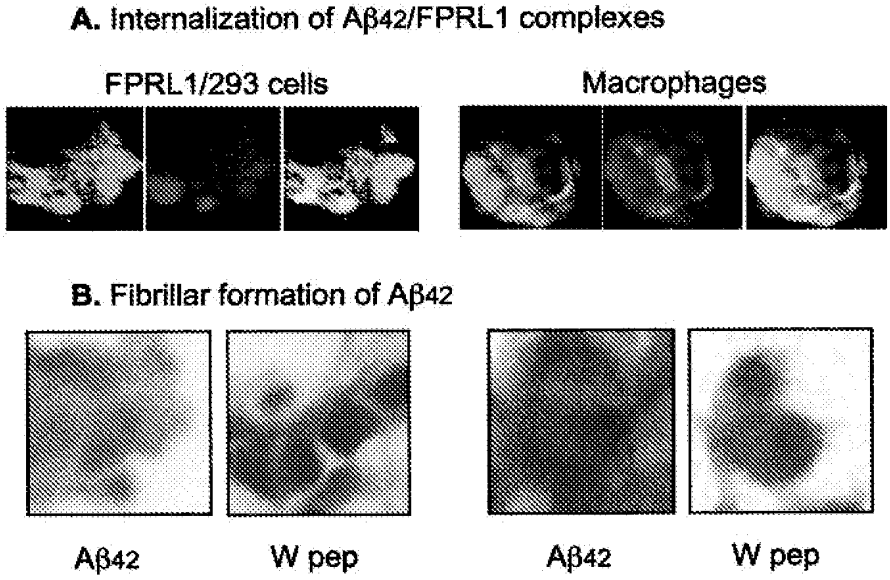

Fig. 2. Internalization and fibrillar formation of $A\beta_{42}$ in monocytic phagocytes. (A) After incubation with $A\beta_{42}$, FPRL1 [green, fluorescein isothiocyanate (FITC)] is internalized into the cytoplamic compartment of HEK293 cells transfected with FPRL1 (FPRL1/293 cells) and human macrophages. $A\beta_{42}$ is detected in red (phycoerythrin) fluorescence and is colocalized with FPRL1 (yellow). Nuclei of the cells are shown in blue (DAPI). Pictures were taken by confocal microscopy after a 24-h incubation period at 37°C with 10 μM $A\beta_{42}$. (B) FPRL1/293 cells and macrophages were incubated with 10 μM $A\beta_{42}$ or 1 μM W pep for 48 h at 37°C. The cells were thoroughly washed, fixed, and then stained with Congo-red and counter-stained by hematoxylin. Fibrillar deposits were detected only in macrophages incubated with $A\beta_{42}$.

plaque formation in elderly and AD patients also implied a role for these cells in transforming diffuse plaques into neuritic plaques [72, 73]. In addition, ultrastructural evidence suggests that mononuclear phagocytes may have the capacity to lay down amyloid fibrils within plaques [74]. This leads to the hypothesis that microglial cells may be involved in the conversion of nonfibrillar $A\beta_{42}$ into amyloid fibrils, a function that was previously ascribed to peripheral macrophages in systemic amyloidosis. In fact, it has been suggested that microglial cells may up-take and internalize amyloid peptides presumably through scavenger receptors [75].

It has been well established that upon binding agonists, STM receptors undergo rapid internalization; we therefore investigated whether FPRL1 also participates in up-take and fibrillar formation of $A\beta_{42}$ in cells expressing this receptor. By using confocal microscopy and a polyclonal antibody generated against the C-terminus of FPRL1, we first studied the localization and trafficking of FPRL1 after incubation with a small peptide [Trp-Lys-Tyr-Met-Val-D-Met (WKYMVm, W pep)] identified from a random peptide library [76] with potent chemotactic activity for FPRL1 [77]. In HEK293 cells transfected with FPRL1 (FPRL1/293 cells), W pep rapidly induced internalization of FPRL1, which reached maximum after 15–30 min treatment at 37°C [78]. When W pep was removed from culture medium after 30 min incubation with the cells, FPRL1 progressively recycled to the cell surface, and after 2 h, most FPRL1 was relocated on the cell surface. These observations established a feasible approach to the evaluation of FPRL1 internalization and recycling by using the agonist $A\beta_{42}$. After incubation for 5 min, $A\beta_{42}$ and FPRL1 were colocalized on the cell surface, followed by a rapid and progressive internalization of the $A\beta_{42}$/FPRL1 complexes. Similar to W pep, $A\beta_{42}$-induced FRPL1 internalization also reached a maximal level at 15–30 min in FPRL1/293 cells and macrophages. At this time point, when FPRL1/293 cells or macrophages were further cultured in $A\beta_{42}$-free medium, the FPRL1 could be detected on the cell surface within 2 h, suggesting an active receptor recycling after depletion of $A\beta_{42}$ from culture supernatant. In the meantime, the antigenic $A\beta_{42}$ was detected in the cytoplasmic region of the cells. Thus, a transient interaction of $A\beta_{42}$ with FPRL1 promotes internalization of the ligand/receptor complexes, and $A\beta_{42}$ was released intracellulary before the receptor FPRL1 travels back to the cell surface. However, a persistent presence of $A\beta_{42}$ in culture supernatant (for up to 48 h) resulted in a massive retention of $A\beta_{42}$/FPRL1 complexes in the cytoplasmic region in FPRL1/293 cells and macrophages (**Fig. 2**). Furthermore, a cytopathic effect was observed as shown by an increase in the proportion of apoptotic cells (**Table 1**). Macrophages incubated with $A\beta_{42}$ for 24 h stained positively with Congo-red, and this staining was markedly intensified at 48 h, suggesting that $A\beta_{42}$ has the potential to form aggregates when it is internalized with FPRL1 in macrophages. In contrast, although massive colocalization of $A\beta_{42}$/FPRL1 could be observed at 24 h and 48 h in FPRL1/293 cells, no Congo-red-positive fibrils were detectable in these cells [78]. It is interesting that W pep, despite its being a potent agonist for FPRL1, did not cause any increased tendency of cellular apoptosis and did not form any detectable Congo-red-positive aggregation in macrophages (**Fig. 2**). These observations suggest two important issues in the mechanisms of amyloid aggregate formation: first, only cells of the

TABLE 1. Cytotoxic Effect of $A\beta_{42}$ on Cells Expressing FPRL1[a]

	% of Apoptotic/necrotic cells[b] (stimulants)		
Cell type	Medium	W pep	$A\beta_{42}$
Macrophages	17.7	17.6	26.2
HEK293	10.3	11.7	11.3
FPRL1/293 cells	9.2	9.6	75.6

[a] Human macrophages, HEK293 cells and HEK293 cells transfected with FPRL1 cDNA, were incubated for 48 h with medium alone, W peptide (W pep; 1 μM), or $A\beta_{42}$ (10 μM) at 37°C. The cells were then examined by flow cytometry after staining with annexin-V-FITC for apoptosis and propidium for necrosis. [b] % Represents a sum of the cell proportions stained positively by either or both markers.

mononuclear phagocyte lineage may provide an appropriate microenvironment favoring fibrillar formation of $A\beta_{42}$, and second, the physicochemical property of the agonist is essential for aggregation in mononuclear phagocytes.

Although the intracellular microenvironment in monocytic phagocytes favors fibrillar formation of the internalized $A\beta_{42}$, the uptake of $A\beta_{42}$ by these cells may also serve to maintain a dynamic balance between amyloid deposition and removal, a process that determines the amyloid burden in AD brain [74]. Cultured rodent microglial cells and human monocytes have been shown to internalize $A\beta_{42}$ peptides [79–81], and $A\beta_{42}$ taken by rat microglial cells could be degraded [82, 83]. These cells also were capable of breaking apart phagocytosed plaque cores [82]. Recent studies provided additional evidence for the capacity of mononuclear phagocytes to remove amyloid deposits. In these studies, $A\beta_{42}$ was colocalized with a microglial activation marker, major histocompatibility complex (MHC)2, in $A\beta_{42}$-immunized PDAPP transgenic mice, in which amyloid deposits were largely cleared [83]. In PDAPP transgenic mice, the AD-like lesions in the brain were mostly of the diffuse type, which is not associated with as prominent a proinflammatory response as seen in the dense, core-type lesions [83]. Thus, the capacity of the host cells, mononuclear phagocytes in particular, to take up and clear $A\beta_{42}$ may be determined by the levels of $A\beta_{42}$ produced and the duration of cell exposure. This concept is supported by our observation that removal of $A\beta_{42}$ from culture supernatants of macrophages after a short period (30 min) incubation resulted in a rapid recycling of the FPRL1 to the cell surface and degradation of the $A\beta_{42}$ dissociated from FPRL1 in the cytoplasmic compartment [78].

CONCLUDING REMARKS

There is considerable evidence for the deleterious effects of inflammation in AD. FPRL1 mediates the chemotactic activity of $A\beta_{42}$ for mononuclear phagocytes and therefore, may participate in the recruitment of such cells at the sites of lesions. In addition, $A\beta_{42}$ bound to FPRL1 is rapidly internalized into the cytoplasmic region as ligand/receptor complexes in mononuclear phagocytes. This process may represent responses of host defense aiming at the clearance of abnormally elevated, pathogenic $A\beta_{42}$. However, the $A\beta_{42}$ interaction with FPRL1 is clearly associated with cell activation [12] and the release of proinflammatory and neurotoxic mediators [28, 35]. In addition, retention of $A\beta_{42}$ in mononuclear phagocytes as a result of persistent internalization of $A\beta_{42}$/FPRL1 complexes culminates in intracellular fibrillar formation and apoptotic death of the cells (**Fig. 3**). In this regard, therapeutic agents that are able to disrupt $A\beta_{42}$/FPRL1 interaction may prove beneficial in the treatment of AD. For instance, NSAIDs, which have been effective on AD prevention and treatment, were shown to block the secretion of neurotoxic mediators by monocytes and microglial cells following stimulation with $A\beta_{42}$ in vitro [28]. One of the NSAIDs, ibuprofen, significantly reduces the proinflammatory responses in brains of the murine AD model and may directly inhibit the aberrant production of $A\beta_{42}$ by neuronal cells [84]. In our studies, another NSAID, colchicine, was found to inhibit $A\beta_{42}$-induced chemotaxis of mononuclear

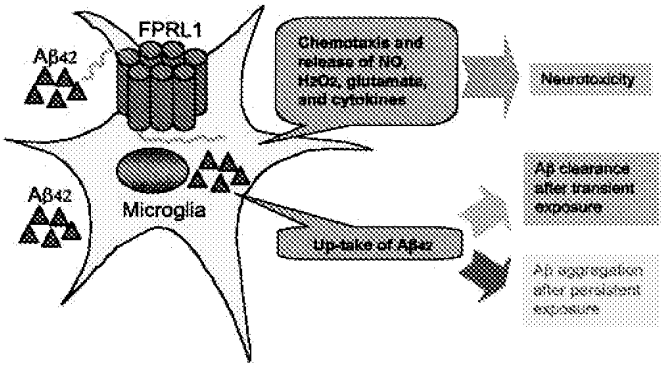

Fig. 3. The putative role of FPRL1 in the pathologic process of AD. Elevated $A\beta_{42}$, by activating FPRL1 on mononuclear phagocytes (microglia) in the brain, increases cell migration (chemotaxis) and release of neurotoxic mediators. FPRL1 also promotes internalization of $A\beta_{42}$. Persistent exposure of the cells to $A\beta_{42}$ results in retention of the $A\beta_{42}$/FPRL1 complexes in the cytoplasmic compartment, which culminates in fibrillar aggregation of $A\beta_{42}$. The expression and function of FPRL1 in microglial cells can be promoted by proinflammatory signals such as LPS and TNF-α.

phagocytes and furthermore, to block $A\beta_{42}$ internalization through FPRL1 and the subsequent formation of Congo-red positive fibrillar deposits, even after prolonged cell exposure to $A\beta_{42}$ [78]. These results suggest that NSAIDs can act at multiple signal transduction levels, including the interference with $A\beta_{42}$/FPRL1 interaction, to exert their beneficial, therapeutic effects on AD. However, unlimited use of NSAIDs for prevention and treatment of AD may cause serious complications in the gastrointestinal tract and kidney as a result of inhibition of cyclooxygenase I [85]. Therefore, efforts should be made to develop alternative drugs, among which FPRL1-specific antagonists may have promising therapeutic potential for AD.

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